NEURONAL PATHWAYS AND SYNAPTIC CONNEXIONS IN THE ABDOMINAL CORD OF THE CRAYFISH*

BY G. M. HUGHES† AND C. A. G. WIERSMA

The Division of Biology, California Institute of Technology, Pasadena, California

(*Received* 20 *October* 1959)

As material for the study of functional connexions within a central nervous system, invertebrates with ganglionated nerve cords appear to offer advantages absent elsewhere. In such animals it should be possible to lead off from the axons which transmit the information from one synaptic region to another at all levels in the connectives, provided there are no cells or synapses in these connectives. In the crayfish, it has been previously shown that such an analysis is indeed possible, at the level between the 'brain' and the remainder of the ventral cord. It was found (Wiersma, Ripley & Christensen, 1955) that many interneurones respond to stimulation of several segments of the body, usually to stimulation of homologous areas of the individual segments. Three fundamentally different ways in which the inflow to different ganglia may become integrated in this way were suggested by Wiersma (1958), and are illustrated diagrammatically in Fig. 1. To gain further information about the occurrence of these different types, it was desirable to obtain recordings from interneurones at a lower level in the cord where the actual integration takes place. The isolated abdomen preparation proved highly suitable for this purpose. The number of fibres in each connective here is still smaller than that in each circumoesophageal commissure (about 1200 as against about 2000), and it was found possible to obtain from the cord preparations in which unit responses were clearly recognizable with not much more difficulty than from the commissure. Altogether it was possible to account for some 75 definite entities as against 100 in the commissure. In both places some of these are not single fibres but bundles of primary sensory fibres with similar properties. In the cord such bundles are more frequent than in the commissure. The nature and distribution of these entities will be described elsewhere; in the present paper their physiological properties will be discussed with special reference to the evidence they provide for the existence of these three possible types of integration. The results will show that the one in which a single interneurone collects impulses from different ganglia (scheme C of Fig. 1) is certainly realized in many of these interneurones.

MATERIALS AND METHODS

Both sexes of the crayfish *Procambarus clarkii* were used. The abdomen was separated from the rest of the animal by a cut between the last pair of walking legs on

* This investigation was supported by Grant G-5461 of the National Science Foundation.

† On leave of absence from the Department of Zoology, University of Cambridge.

the ventral side and the posterior edge of the carapace dorsally. The isolated abdomen was fastened ventral side uppermost by pins through the first abdominal segment in a dish containing ice-cold crayfish solution. The chain of abdominal ganglia was next exposed by removing a strip of cuticle from the mid-ventral portions of segments 2–5. Care was taken not to damage any of the three pairs of nerves from any of these ganglia. The first pairs of roots are especially vulnerable as they run close beneath the rigid sternum of each segment. The cord was usually prepared for splitting between the third and fourth ganglia by removal of the sternal blood vessel and by placing a small piece of black film beneath it. The sheath surrounding the

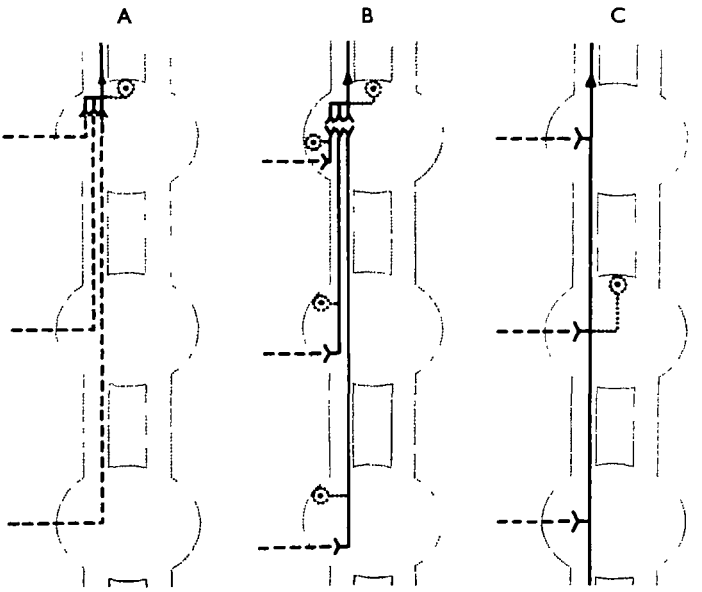

Fig. 1 Diagram to show three possible types of neural connexions which would result in a single interneurone firing when sensory areas of three separate segments were stimulated. Dashed lines show primary sensory fibres; dotted lines indicate the cell bodies of the interneurones, whose locations are unknown, full lines interneurones. Synapses in which several pre-synaptic fibres converge on a single post-synaptic fibre are shown: - - -›—, whereas a synapse between only two fibres is shown: – –‹›—. The same conventions are adopted in Figs. 3, 6, 8 and 9. For further explanation see text.

connectives was split horizontally, and this section was extended from just in front of the fourth ganglion to the region where the third roots of the third ganglion leave the cord. As in previous work with the circumoesophageal commissures, these and all subsequent splits were marked on a schematic outline of a cross-section so that the location of a bundle under investigation was approximately known (Wiersma, 1958). Leading off was first done 'biphasically', i.e. by lifting the bundle on a single electrode just above the surface of the fluid which was grounded. Subsequently, depending on the type of response from it, the bundle was further divided or the lead was made monophasic by cutting the bundle and lifting it out of the fluid. After a short drying-out period, monophasic responses were then obtained. The lead was

taken either from the anterior or posterior part of the bundle, or in some instances from both at the same time with the use of a second electrode. In general, only those bundles were used in which the main response gave proof of being definitely due to a single unit on the oscilloscope and through the loud-speaker.

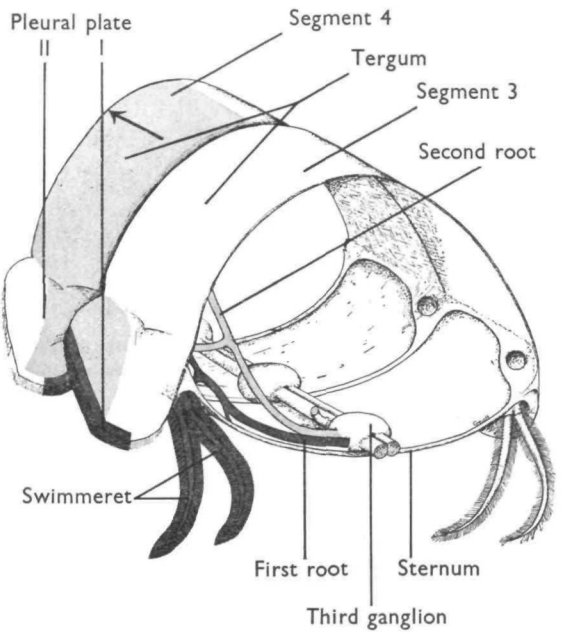

Fig. 2. Stereogram of the third and fourth abdominal segments of a crayfish showing the sensory innervation of the right half of the third ganglion. First root's fields: dark shade; second root's fields: light shade. Arrow indicates the position of the right fast and slow abdominal stretch receptors for the joint between the fifth and fourth segments.

Mechanical stimulation in all experiments in which touch of hairs was investigated was done by touching different parts of the exoskeleton with a fine brush, and if necessary for more precise localization of the stimulus area, by a bristle or needle. Joints were moved by manipulation with needles and sometimes manually or by reflex contractions. Care was taken to localize the stimulus as much as possible to one area or joint. In some cases this was extremely difficult because of the high sensitivity of certain end-organs which responded, for instance, as soon as the meniscus of the fluid was disturbed anywhere in the dish. Nomenclature used to describe the areas which excited a given fibre when stimulated is shown in Fig. 2.

Most preparations were used for periods up to 4 hr. There is a slow but continuous deterioration in such preparations. As in the commissure the primary sensory fibres remained responsive longer than the interneurones, though the latter often persisted for several hours. In most preparations the first abdominal ganglion was unresponsive from the start but the sixth ganglion, although also less exposed than the others, did not obviously differ from them in its reactivity. During the

later stages the interneurones failed first to react to stimuli reaching them through the second ganglion, whereas the other ganglia became affected later all at about the same time.

RESULTS

The units from which recordings were made can be classified into the two major groups of primary sensory fibres and interneurones. In most instances this distinction was easily made, since the majority of primary sensory fibres respond to more localized stimulation, with higher frequencies and with smaller impulses, whereas the interneurones, in addition to being responsive to stimulation of a larger area and having impulses of greater recorded amplitude and lower frequency, often show more rapid fatigue and other 'central' features such as inhibition and facilitation.

(*A*) *Primary sensory fibres*

As stated above, these form a far greater portion of the fibres in the abdominal connectives than they do in the circumoesophageal commissures. They enter the cord through the first and second roots of each segment, the third roots being entirely motor (Hardy, 1894; Wiersma, 1947*a*). The two roots supply quite distinct sensory areas and it is convenient to consider each of them with its central pathways separately.

(i) *First root areas.* Sensory fibres from hairs and joints of the swimmerets enter via this root, together with hair fibres from the ventral abdominal surface as well as those from the distinct tuft of hairs on the lateral edge of each segment (pleural plate I, Fig. 2). In this figure it can be seen that all fibres from a given swimmeret enter the ganglion situated in that segment, but that the sensory inflow from hairs of the anterior third of pleural plate I enter the ganglion next to the anterior. In the cord between the third and fourth ganglia, primary fibres ascending from the fourth and fifth segments and descending from the third segment are found which respond to movements of the swimmeret joints. These are of various types depending for activation on the direction of movement and differing also in rate of adaptation. They are not restricted to basal joint movements but are also found for the more distal joints of the swimmerets. In some cases the nature of the discharge which occurred mainly or only during active movements suggested that they might be due to the type of ending described by Alexandrowicz (1958) in the coxal region as 'muscular receptors'. All sensory fibres appear to form distinct bundles, in which those of similar origin run together. There is definite evidence that the bundle of swimmeret joint fibres ascend through at least two ganglia, but they do not seem to descend through more than one. The bundles of primary hair fibres from the swimmerets and from the pleural plates spread much less from their place of entry into the cord, solely descending to the next ganglion.

(ii) *Second root areas.* Through this root enter the well-known large primary fibres from the stretch receptors in the abdomen. These have also been found in the circumoesophageal commissures. They are recognizable by their characteristic discharge on flexion of individual abdominal joints. Any doubt that might exist about

their primary nature once they have entered the cord could be removed in the present experiments by recording simultaneously from the second root and from the fibre dissected in the abdominal cord. Identical records except for a small difference in the arrival times were obtained. Their conduction speed is about 8 m./sec.

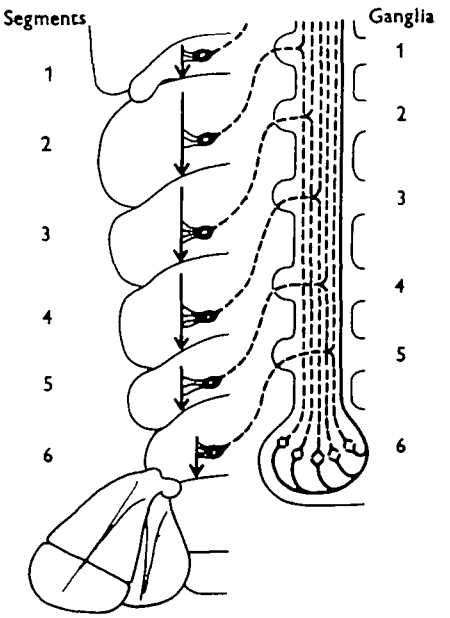

Fig. 3. Diagram of the path of sensory fibres from the homolateral slowly adapting muscle receptor organs in the abdomen. One half of the cord, from the first to the sixth ganglion, is shown. Primary sensory fibres are indicated by dashed lines, the interneurone of the sixth segment by a solid line (see text). Arrows indicate the joint which when flexed excites a given receptor.

Two unexpected features were established concerning the course of these fibres. In the first place, the second root in which they enter the cord is one segment more anterior to the segment whose posterior joint they serve. For example, fibres from the sense organs signalling flexion of the hinge between the fifth and the fourth abdominal segments enter via the second root of the *third* abdominal ganglion (Figs. 2, 3). Secondly, not only do they send out an anterior branch as is necessary to account for their presence in the commissure, but they also have a posterior branch which ends in the sixth ganglion. Thus it is possible to lead off impulses both in front and behind a given ganglion, which are again identical with respect to frequency. These experiments clearly demonstrate that transmission at their T (or Y) junctions in the ganglion is equal in both directions. As expected, it was found that these responses are completely homolateral, the fibres running up and down the cord at the same side at which they enter (Fig. 3). From a few experiments in which the two fibres of the slowly adapting organs of one segment were both prepared, it was evident that the responses were very much alike in overall frequency but were not identical in their detailed pattern. The central branches of the stretch

receptor fibres form a very definite bundle, both in the commissure and in the cord. In the latter the ascending and descending branches are mixed. This bundle is located ventral of the median giant fibre in both places.

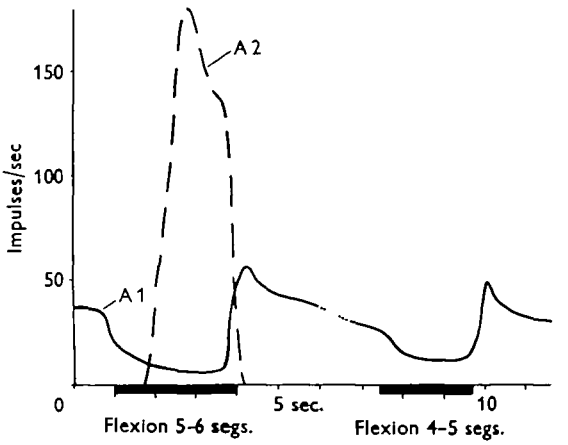

Fig. 4. Plot of impulse frequency in two fibres of an abdominal connective between the third and fourth ganglion during flexion of: (*a*) joint between fifth and sixth segments, (*b*) joint between fourth and fifth segments. A 1, tonic extensor fibre; A 2, slowly adapting fibre of the joint between the sixth and fifth segments.

In this same bundle other fibres were found which were stimulated by the opposite movement, i.e. tail extension. One of these (A 1 of Fig. 4) discharges tonically even when the tail is in the position which it takes up when no forces act on it other than the very mild one of gravity on the unsupported telson. By leading off at the same time from one of the tonic stretch receptors (Fig. 4, A 2) it can be shown that for flexion of this segment there is a reciprocal relation of the discharges; flexion of another segment will, of course, not affect the same stretch receptor, but does have a similar inhibiting effect on the extensor fibre. In correspondence with this latter finding, the frequency of the extensor response can be increased by extension of any of the joints between posterior segments. Complete and lasting stopping of firing occurs only when all of them are kept flexed. Many attempts were made to locate the sense organs involved in these discharges. All roots of different abdominal ganglia have been investigated, but in none of them were such signals found, and in the cord they persisted when all first, second or third roots were cut, also in combination. It is known (Wiersma *et al.* 1955) that in the commissures certain fibres may be brought to discharge by stretching the axon with the leading-off electrode. By preparing long lengths of the cord fibres, which allowed for slack even when the fully stretched position of the tail was reached, this possibility was here excluded, the response taking place as readily in the slack nerve fibre. But this does not exclude the possibility that stretch of the axon is the stimulating factor in the sheathed part of the cord. Another possibility is that these fibres have dendritic processes in the cord sheath and signal its stretching. Whether the fibres themselves are primary or

secondary is unknown, though they do signal stretch of more than one segment. It should be stressed that there are three such fibres, which differ in their properties, one being phasic, one tonic and one intermediate, and that all three have been found several times in the same preparation. This constancy of response patterns and the constancy in their location make it highly likely that the perception of abdominal stretch is their true function and not an artifact.

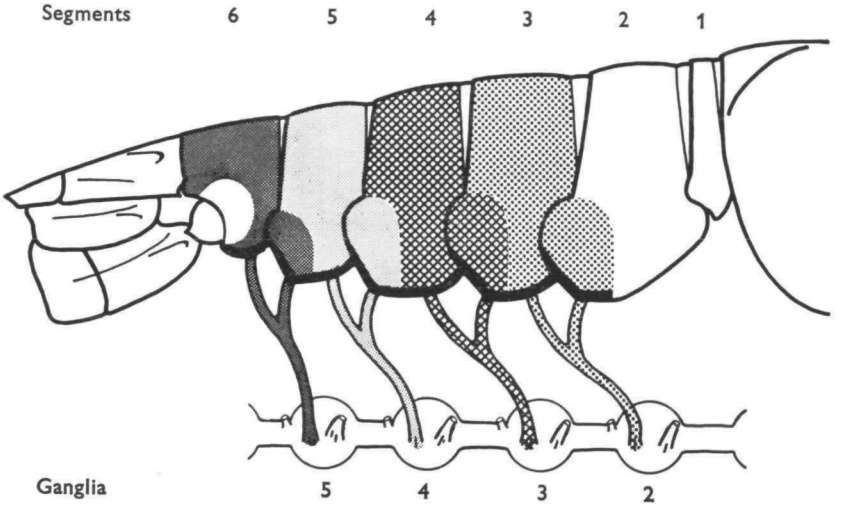

Fig. 5. Diagram to show the peripheral pathways of sensory fibres from hairs on dorsal abdominal segments which enter through the second roots of the second to fifth abdominal ganglia. Note the slant of the neural segment as compared to the skeletal one.

The hair fibres from the sensory areas on the dorsal side which enter through the second root are also mostly from the segment posterior to that containing the ganglion. As shown in Figs. 2 and 5, the sensory input from the same segment comes only from a small part of the posterior pleural region, whereas that from the next posterior segment comes from the whole dorsal area except for a corresponding pleural area. In the cord such fibres form broad bundles in which those of adjoining segments are associated. They are both ascending and descending. Whether or not the descending fibres are all only from the dorsal area of the posterior segment and not from the posterior lateral area of the same segment is not known.

(*B*) *Interneurones*

As in the commissures, interneurones were found which reacted either to touch or to proprioceptive stimuli and a few to both. Depending on whether the stimulation was effective on the same side of the body in which the interneurones run, or on the other side or both, they can be distinguished as homolateral, heterolateral or bilateral interneurones.* In each of these classes, interneurones are present which react on stimulation of a single abdominal segment or of several consecutive segments.

* Some asymmetrical fibres which include both homolateral and heterolateral areas, but not symmetrically divided, are also present, but will not be further discussed here.

Quite a number of fibres have been found which apparently only respond to stimulation of dorsal hairs on a single segment, which had not been encountered in the circumoesophageal commissure. Most of these are homolateral but similar heterolateral fibres have also been obtained. In contrast to the primary sensory fibres, responses can be obtained by stimulating quite different places on the dorsal surface and they have a distinctly higher 'threshold', e.g. do not readily fire with a slight touch of a brush and show a much lower frequency of response. Interneurones excited by afferents in the first root of a single segment have also been found. It is difficult, however, to distinguish those which react to proprioceptive

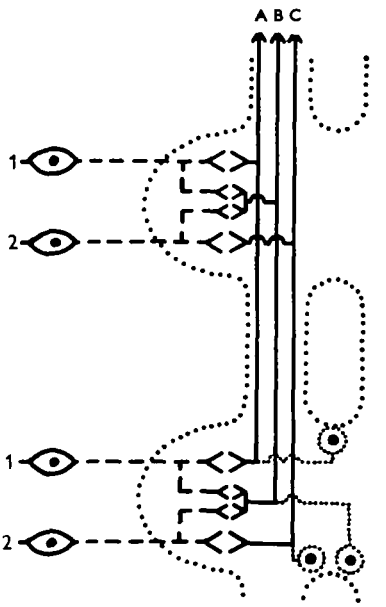

Fig. 6. Diagram of three different interneurones, two of which (A, C) respond to stimulation of two distinct types of sensory field (1, 2) on several segments, whereas type B is excited by stimulation of both of the areas which A and C innervate.

function of the swimmerets from the similar primary sensory fibres, since the latter may also give rather low frequency responses, and 'areal' stimulation is almost impossible because of the structure of the swimmeret. Interneurones responding to only two segments, which are then always consecutive, are present for both homolateral dorsal and heterolateral dorsal areas, but not for ventral ones. There are also some very good examples of fibres responding to stimulation of three or more dorsal segments as well as for fibres responding to several ventral segments or to both dorsal and ventral parts combined. Several of these are identical in their properties with those found in the commissure.

Like single-segment interneurones, those responding to several segments may do so only to a specific part of each segment, for instance, the hairs on pleural plate I. A fibre responding to two segments in this way is represented by 'A' in Fig. 6.

Fibre 'C' represents a similar one of this type, e.g. one responding to hairs on the swimmerets. In addition to these fibres which collect more detailed information, fibres are present which are connected like 'B', in this example thus sensitive to hairs on both the pleural plate I and the swimmeret.

The fibres responding to homolateral sensory fields in three or more segments offer the best opportunity to test for the three possible arrangements shown in Fig. 1. In all of these interneurones tested with a 'diphasic' lead in the connective between the third and fourth ganglion, it was found that impulses passed in both directions when the extent of their sensory fields was such that this was to be expected. Descending impulses could be observed on stimulation of anterior fields, ascending ones on posterior stimulation. After cutting and applying a lead to each end, the two leads only record impulses from the appropriate fields. It is essential, of course, to know that all reactions do take place in the same interneurone. In the 'diphasic' lead this is often very clear at the beginning of an experiment, whereas in later stages there may develop a difference in shape between descending and ascending impulses, due to a block in the area of the electrode. We have therefore used other ways of recording as well, in order to substantiate this observation. As collision of impulses must occur, 'diphasic' recordings were made from the same small bundle with two electrodes at about 6 mm. distance. The direction of the impulses was then clearly indicated by the lead in which they first appeared. Again, unequivocal evidence was found in many preparations that impulses pass in both directions according to the areas stimulated. With simultaneous stimulation of areas, evoking impulses travelling in different directions, it has, in a few cases, been possible to prove that when two impulses arrive simultaneously at the two electrodes, neither of these reaches the other electrode, proving that they, in contrast to all others, collided *between* the electrodes (Fig. 7). This type of observation has been made for interneurones excited by sensory inflow from either first or second roots, but for many purposes those excited by the joints of all homolateral swimmerets were the most convenient as the stimulation was more easily localized and the discharges in some of these fibres were distinctly tonic.

In contrast to these homolateral interneurones, some of the bilateral ones show the interesting feature that after cutting them between the third and fourth ganglia they still respond in the anterior recording to all the homolateral and heterolateral sensory fields to which they were sensitive in the 'biphasic' recording, and thus to segments of the abdomen both in front and behind the leading-off position. Furthermore, this can also obtain for the posterior lead. These observations make it necessary to postulate central connexions of the type D shown in Fig. 8. For such a fibre it is necessary to suppose that it makes connexion with its homologue on the other side in each ganglion, a situation known to exist for the lateral giant fibres (Wiersma, 1947*a*; Furshpan & Potter, 1959). If these connexions are fairly labile, different types of results are to be expected depending on which junctions fail to transmit. Such variations have been found between fibres which were identical in their location and sensitive fields. A special case of this is shown in scheme E of Fig. 8, for which there is evidence that it may occur naturally in a special fibre, but in other

cases was apparently due to the loss of junctional transmission in all but the most anterior cross-connexions. This type E pathway explains the cases, which have been rather frequently encountered, where the fibre only responds on the homolateral side behind the cut in the posterior lead, whereas the whole remaining part of the receptive field including the posterior heterolateral areas produce responses in the anterior lead. Note that on the homolateral side such interneurones are excited directly by sensory fibres, but on the heterolateral side by an interneurone.

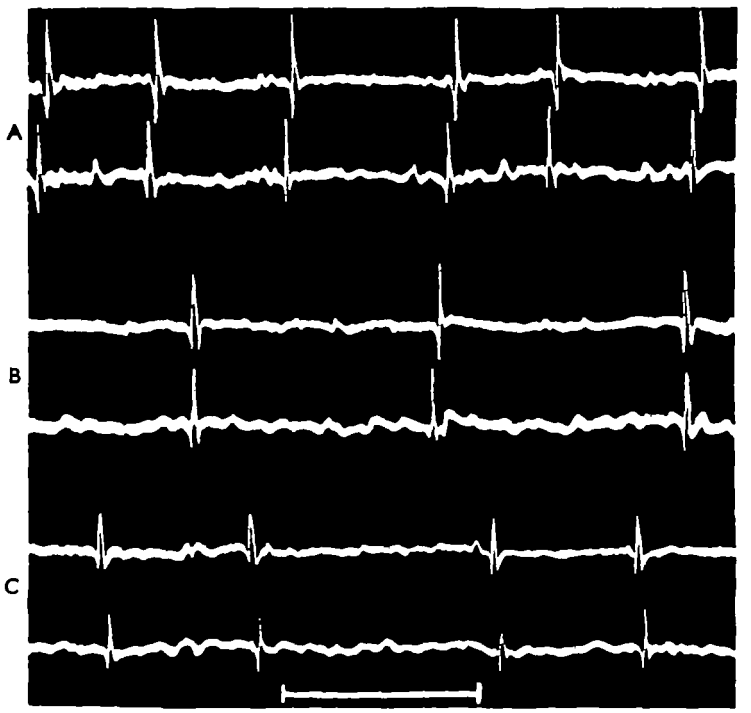

Fig. 7. Three records from two 'diphasic' leads from a small bundle of fibres in a 3–4 abdominal connective In each frame the upper of the two lines is from the anterior lead. Stimulation in upper frame from posterior segment, in lower frame from anterior segment, and in middle frame from simultaneous stimulation. The first and last impulses in the middle frame collide between the leads. Cuts from long records. Note the gradual reduction of action potential size between frames which developed with time. The spikes are retouched. Time $\frac{1}{10}$ sec.

Some other bilateral fibres do, however, invariably respond in a way analogous to that described above for homolateral interneurones, being excited in the anterior lead only by the bilateral sensory fields above, and in the posterior ones by those below the lead. It is here necessary to postulate a pair of interneurones which do not make mutual synaptic connexions, but which in each ganglion are stimulated by sensory fibres from both sides of the body (scheme F of Fig. 8). There are two alternatives with regard to the way in which crossing in the ganglia occurs. Either primary sensory fibres from the heterolateral side may decussate to make connexion

with the interneurone, or the latter may send a collateral to the neuropile of the other side. From what is known of the histology of crustacean ganglia both alternatives are possible.

This same question arises with regard to the heterolateral interneurones, several of which are found to be responsive to stimulation of dorsal hairs of successive abdominal segments. In most of these the impulses appear to arise in either one or the other side of the lead, and are thus explained by scheme G of Fig. 9. However, it was found that a fibre for the fifth heterolateral dorsal segment gave descending as well as ascending impulses in a lead between the second and third ganglia. This indicates connexions of the type shown in scheme H of Fig. 9, in which the heterolateral unit is a secondary interneurone.

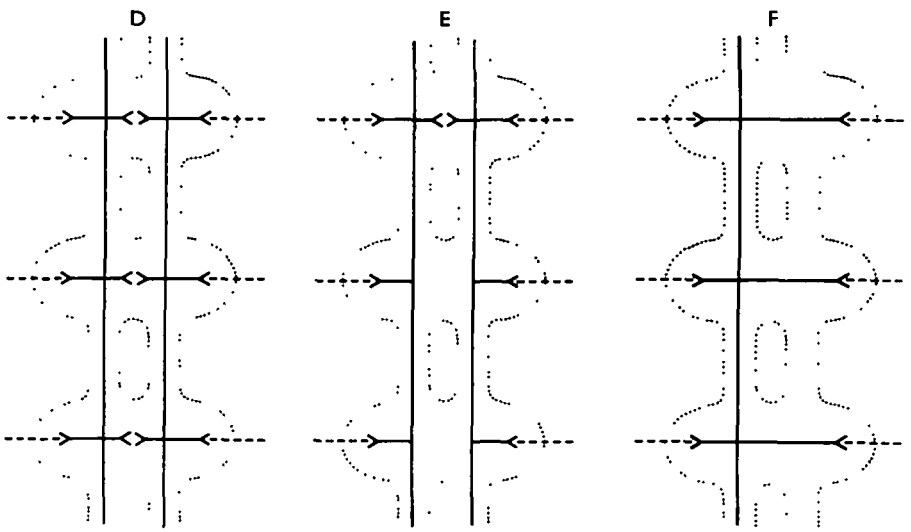

Fig. 8. Three different types of connexions (D, E, F) indicated by the findings in interneurones responding to bilateral sensory stimulation. For explanation see text.

The behaviour of all interneurones discussed above which respond to stimulation of receptive areas on three or more segments, whether these be homolateral, bilateral or heterolateral, strongly suggests that without exception they can be excited in each ganglion and are thus of type C (Fig. 1). But this is not the only type realized, for clear evidence has been found for type A (Fig. 1) connexion in at least one interneurone. This fibre integrates the inputs from all of the primary sensory fibres of the slowly adapting muscle receptor organs on one side of the abdomen. (This, strictly speaking, is not proven, as the most anterior ones of these, abd.segm. 1-thorax, and abd.segm. 2–1 could not be tested in the preparations made.) As can be seen in Fig. 3, the region where this integration occurs is in the last (sixth) ganglion and the fibre is thus activated by descending impulses in contrast with the situation pictured in Fig. 1 A. The final experiment on which this fibre was established as a unit in the cord was made possible only because of the previously acquired know-

ledge of the primary sensory pathways. In this experiment the fibre, integrating all tonic stretch receptors, was found at a time when all the descending branches of the latter were still intact. The interneurone was found to respond therefore as expected, to both flexion of the fifth on the fourth abdominal segment, as well as to that of the sixth segment on the fifth. By cutting the bundle containing the primary sensory fibres in the connective between the third and fourth ganglia, it would follow that the reaction to the first-mentioned stimulus should disappear (since the stretch receptor axon enters the second root of the third ganglion) but that the other should remain. This actually was found, and since the heterolateral connective was intact, it also followed that the interneurone was a homolateral one. These experiments also indicate that the integration must take place either in the fifth or the sixth ganglion. The latter appears the much more likely possibility, as histological evidence indicates that the descending branches of the primary muscle receptor fibres do not stop in the fifth but run through to the sixth ganglion.

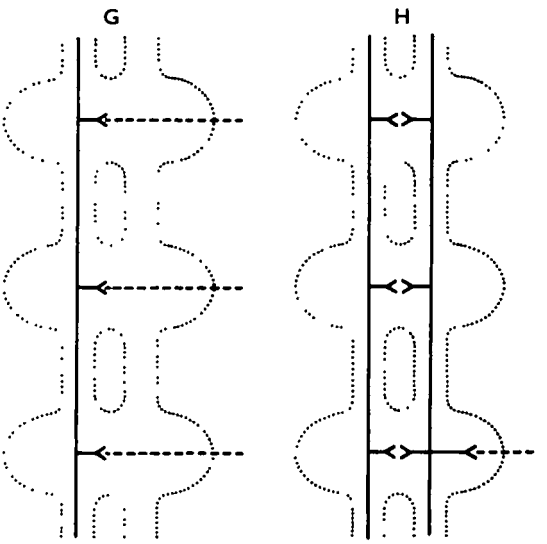

Fig. 9. Two types of connexions, (G, H) of heterolaterally responding pluri-segmental interneurones, as indicated by experimental observation. For explanation see text.

Direct evidence for the existence of type B (Fig. 1) pathways has not been obtained in the present work, but its possible presence is indicated by the finding of many interneurones which respond to sensory areas of only one segment. Such fibres were not found, except for the first abdominal segment, in the commissure. It is therefore possible that some of these lead to integration in a more anterior ganglion and form some of those interneurones in the esophageal commissure which, in contrast to others, have not been found in the cord.

DISCUSSION

The methods used in the present work have given useful information not only concerning the types of synaptic connexions which exist between different primary sensory fibres and interneurones, as presented in this paper, but also concerning the functions and pathways of a number of individual neurones, which will be presented elsewhere. For both aspects histological data are complementary but unable to answer the pertinent questions by themselves. For the present purpose it should be noted that it appears impossible with available techniques to trace the connexions which primary sensory fibres make with specific interneurones in the neuropile. From our results it is quite clear that such connexions follow very definite rules, e.g. one interneurone making connexions with one part of the sensory inflow, another with a different part, and a third with both (Fig. 6). This type of finding requires the precise development of all these connexions, a picture greatly different from that which is obtained from the histological one in which there appears little of this type of order in the neuropile.

Whereas in this respect histological findings are as yet of no help, in other cases they complement the physiological results and confirm the presence of certain elements at an anatomical level. For instance, in Allen's Figs. 11 and 12 (1894), rather large fibres are shown which enter the second root of an abdominal ganglion in the lobster and then form a T-junction with ascending and descending portions, but without a branch into the ganglion it enters. Such a fibre is exactly of the sort that would explain the functional pathway found for the primary sensory fibres of the abdominal muscle receptor organs. Furthermore, Allen's observations on the embryonic lobster have been confirmed and extended by Alexandrowicz (1951) who was able to show that these fibres arose from peripheral cells which are almost certainly precursors of the muscle receptor organs. Our results, made independently and by another technique, completely confirm Alexandrowicz's finding that these fibres form a distinct tract which lies ventral and medial to the medial giant fibre in the connectives. All these observations indicate that such fibres do not have a local reflex arc within the abdominal ganglia, but would excite structures in the upper and lower portions of the central nervous system. For the latter we have direct evidence by finding an interneurone which is excited by all slowly adapting organs, whereas the absence of interneurones stimulated by these fibres in the abdominal ganglia (2–5) may support the idea that they do not make connexions within their neuropiles.

Again, Retzius (1890) shows primary sensory fibres which enter by the second root and immediately descend to another ganglion, and we obtained responses from primary sensory hair fibres of the dorsal abdominal segment which descended. Previously most primary sensory fibres of the roots do not appear to have been traced to their origin. As has been shown, the roots do not innervate the periphery according to the external segmental divisions. Instead, the neural segment slants in a dorso-posterior direction (Fig. 5). The motor axons in the second root innervating the dorsal extensor musculature show a similar distribution (Hardy, 1894).

Hardy considered that displacement of the dorsal muscles is the reason for the shift, and noticed that the blood supply does not take part in it. From our observations on the sensory innervation it is clear that the whole neurotome participates; the motor fibres of the dorsal muscles to a greater extent, since they penetrate into the next posterior segment (Wiersma 1947*b*).

Histological information concerning the different types of interneurones is more difficult to interpret because here practically all quantitative data about the numbers present of the different types described are lacking. Bethe (1897), however, has noted the presence of several interneurones which have connexions similar to those suggested diagrammatically in this paper, including some bilateral fibres of type F (Fig. 8). He also shows fibres which are essentially type E but the transverse connexion between the two sides is asynaptic. These and some unilateral interneurones have segmentally arranged synaptic sites, a type for which there is now abundance of physiological evidence. The existence of this type of connexion (scheme C of Fig. 1) is one of the main conclusions of the present work, but as stated above, types A and B certainly cannot be excluded. It is of interest to consider the different physiological properties to be expected from these three systems and the functions they might serve in the central nervous system.

Type A is the normal one considered to account for the integrative action of interneurones excited by sensory stimulation. In this case it is the properties of the synaptic connexion which will determine how this integration takes place. Variations of threshold and excitability in the synaptic regions will result in interneurones with quite different properties, such as those very sensitive to a few sensory impulses but with quick adaptation, and those which fire at low constant rates to low frequency inputs. During our investigation we have noticed very significant differences between interneurones in these respects (see also, Preston & Kennedy, 1958). In addition the picture can be complicated by the possibility of inhibitory innervation at the same locus. In the abdomen very few fibres have been found which clearly require such a mechanism, though the possibility that it is more widespread is not excluded. The interneurones involved were stimulated either by sensory fibres from hairs or joint organs. The evidence from the former is the more striking. For such fibres, in sharp contrast to most others, the mapping of the sensory area, limited to one segment, is quite easy because touch of surrounding areas inhibits any low frequency discharge caused by slight stimulation of the area.

In type B integration the difference from type A is that two synapses have to be passed. Depending on the synaptic properties of these two, the output in the secondary interneurone could differ very considerably between different interneurones. This arrangement would seem particularly suited for integration of different modalities, but there is no evidence that this is the case. On the contrary, the interneurones known to respond to different modalities appear to be primary interneurones making synaptic connexion with the different kinds of primary sensory fibres.

Some of the properties of connexions of type C have been partially discussed (Wiersma, 1958). Obviously the 'local sign aspect' of the stimulus is increasingly

reduced when additional numbers of segments are involved. Since several types of axons are found in this class, differing in the same way as pointed out for type A interneurones, the consequences for impulse transmission to the 'receiving higher centre' will be considerably different for these reasons. In the type of axon which fires at low frequencies only, the summation aspects will preponderate. For if in the different ganglia only low-frequency impulses are set up, the chances that they are blocked by 'antidromic' impulses diminishes. Of course, the conduction speed of the impulses and the distance between the synaptic areas are also involved. On the other hand, in those interneurones which regularly carry high-frequency signals, occlusion of almost all of the impulses from the receptive areas farthest removed from the 'receiving centre' must be a regular occurrence during simultaneous stimulation. If the centre be the brain, it would follow that more impulses would reach it when quick successive stimulation took place from back to front than from front to back, since it would always be the area closest to the receiving centre which would have the best chance to 'squeeze' impulses in the trains coming from the remoter ones.

There is as yet no evidence whether the type of integration found in interneurones which collect in different ganglia can also occur in the branches of such interneurones in single ganglia. Interneurones are present which collect from more than one of the neuropiles of a single ganglion (Allen, 1894; Bethe, 1897), which may indicate that there is also within a ganglion more than one site at which action potentials can be generated. This arrangement will cause collision of impulses instead of summation of prepotentials. A considerable strengthening of this possibility may be seen in the fact that with internal electrodes two spikes of different shape have been reported to occur in single cells of the sixth ganglion (Preston & Kennedy, 1958). Bullock & Terzuolo (1957) have recorded independent action potentials in different parts of crustacean heart ganglion cells, showing that, here too, more than one locus for spike generation is present in a single cell. It is interesting to note that in this type of system, slight shifts in position of the site of spike generation could change its properties considerably. If this site is close to the neuropiles, each would be able to set up spikes which would mutually collide. But if the site were further removed, the local potentials of the neuropiles would summate and the frequency of spikes would be proportional to the excitation of both combined. The long distances between ganglia precludes the latter mechanism from playing more than a minor part in interganglionic integration.

From a comparative point of view it is likely that the three types of interneuronal connexions discussed here exist in many central nervous systems. In practically all groups of animals histological evidence is available for the presence of interneurones having synaptic regions in spatially separated neuropiles. Although such pathways could function in a variety of ways, it is probable that in many instances the properties described for type C connexions in the crayfish must be considered when assessing their mode of action.

SUMMARY

1. An investigation has been made into the function and distribution of nerve fibres in the abdominal ganglion chain and its roots in the crayfish, *Procambarus clarkii*, by leading off action potentials from small prepared bundles following sensory stimulation.

2. The sensory fields belonging to the first and second roots of each abdominal ganglion were determined, and the antero-posterior pathway of sensory fibres within the cord noted. It was found that the primary sensory fibres of the dorsal muscle receptor organs, entering through the second root, send out an anterior branch to the brain and a posterior one to the last ganglion. For most other sensory fibres much shorter intracentral branches are indicated, though some of them extend for two ganglia in the anterior direction and for one posteriorly. All sensory fibres in the connectives run on the same side as they enter.

3. The segmental divisions of the external skeleton and of the nervous system do not coincide, the neural segment slants in a posterior dorsal direction with respect to the skeletal one.

4. For the majority of the interneurones which innervate more than two abdominal segments it has been proved that they synapse with primary sensory fibres in each of the ganglia that these enter. Depending on the segment stimulated with respect to the leading-off position, both ascending and descending impulses are obtained in such interneurones and collision of the impulses has been observed. Some consequences of this type of integration are discussed.

5. For interneurones responding to bilateral or heterolateral stimulation the course of the impulses proved to be of at least two types. In some, cutting the fibre prevents the arrival of impulses except those set up on the side of the cut from which the recording is made. In others, recording from either side of the cut fibre does not exclude any of the sensory fields to which the fibre normally responded.

6. At least one interneurone is present in which all primary sensory fibres from the different segments to whose activity it responds collect in one ganglion.

We want to thank Mr G. Hickey for his help with the diagrams and other aspects of this investigation and Dr R. Pilgrim for study of certain aspects of the sensory innervation of the abdominal roots, which have been incorporated in the results.

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